



## Instructions for Use: Thawing CELLvo™ Human T-Cells

### CELLvo™ Human T-Cells (TCL)

StemBioSys Cat. No. TCL-5MIL-000 and TCL-10MIL-000

#### Product Description

CELLvo™ T-Cells have been isolated from human buffy coat using negative selection leaving the CD3+ T cells untouched and ready for research applications such as CAR-T research, immunology, and oncology.

These cells have a purity of  $\geq 85\%$  CD3+ expression as measured by flow cytometry.

Each cryovial contains  $\geq 5$  or 10 million viable cells cryopreserved in BioLife® CryoStor CS10 Freeze Media. CELLvo™ T-cells are procured from single donors and never pooled.

#### Quality Control and Stability

Quality control tests are performed on each StemBioSys® CELLvo™ T-Cell lot. Cells are collected from healthy donors that have tested negative for HIV, HBV, and HCV.

All cells have been tested for the absence of mycoplasma (conditioned media) and endotoxin (cryopreserved cells).

A detailed Certificate of Analysis may be requested at [info@stembiosys.com](mailto:info@stembiosys.com) by referencing the purchased lot number.

Upon arrival, store the cryopreserved cells in liquid nitrogen vapor or thaw and seed immediately. Store frozen in liquid nitrogen vapor. Annual stability testing by StemBioSys will be performed while inventory exists. Short-term storage of cells (< 1 month) at  $-80^{\circ}\text{C}$  is acceptable but should be minimized to ensure maximum stability. Thawed samples must be used immediately. As these are primary cells, they have a finite lifespan in culture.

#### Intended Use

All StemBioSys CELLvo™ Products are intended for Research Use Only and should not be used for diagnostic or therapeutic use.

#### Warnings and Universal Precaution

All products of human origin should be handled as potentially infectious. Use appropriate Personal Protective Equipment (PPE) when performing cell culture.

#### Directions for Thawing

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#### Prepare Reagents and Biological Cabinet

Prior to transferring reagents and cells, disinfect the biological safety cabinet with 70% isopropyl alcohol or any other suitable disinfectant.

#### Thawing Cells

Note: Once thawed it is important to work quickly to ensure high viability and recovery. Use sterile techniques when processing thawed cells.

**Culture Media:** Depending on the application, there are many choices for T-Cell culture media. StemBioSys routinely uses and recommends RPMI 1640 Media with 10% fetal bovine serum (FBS). The inclusion of FBS in culture media may be undesirable for certain applications.

1. Warm media to 37°C in a sterile container.
2. Remove the cryovial of cells from liquid nitrogen storage.
3. Disinfect the outside of the cryovial with 70% isopropyl alcohol (IPA). Transfer to a pre-disinfected biological safety cabinet and loosen the cap to release any pressure buildup. Replace the cap.
4. Submerge cryovial in a water bath at 37°C until just thawed (1-2 minutes). Do not submerge the cap or allow water to enter the threads of the cryovial.
5. When just melted, remove cryovial from the water bath and pat dry.
6. In a laminar hood, wipe the outside of the vial with 70% IPA.
7. Transfer the cell suspension to a 50 mL conical tube.
8. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 50 mL tube.
9. Wash by adding 10 or 20 mL of prewarmed medium dropwise while gently swirling the tube. The volume is dependent on the number of cells.
10. Centrifuge the cell suspension at 300 x g for 10 minutes at room temperature (15 - 25°C).
11. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
12. Resuspend pellet in 3-5 mL of media for cell counts.
13. Perform a cell count using a 1:2 ratio of your cell suspension to Trypan Blue.
14. Gently add an appropriate volume of medium to the conical tube, depending on the size of the flask where you will transfer your cells.  
*Note:* The number of cells you will seed will depend on the application.
15. Cells are now ready for use in downstream applications.

Disclaimer: StemBioSys cannot guarantee the biological function, or any other properties associated with performance of cells in a researcher's individual assay or culture systems.

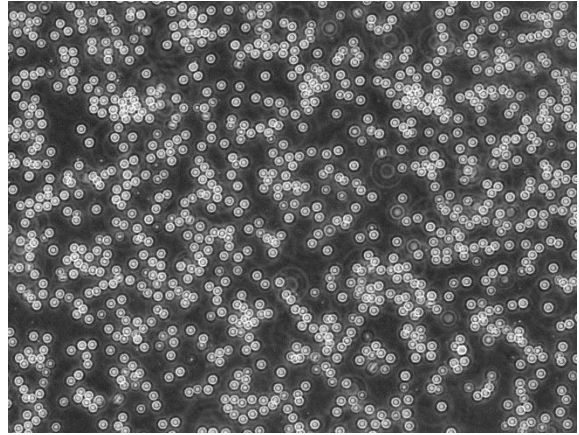


Figure 1. CELLvo™ human T-Cells in suspension

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Information about ordering:

SUPPLIER No.	DESCRIPTION
TCL-5MIL-000	Vial of 5 million CELLvo™ T-cells
TCL-10MIL-000	Vial of 10 million CELLvo™ T-cells